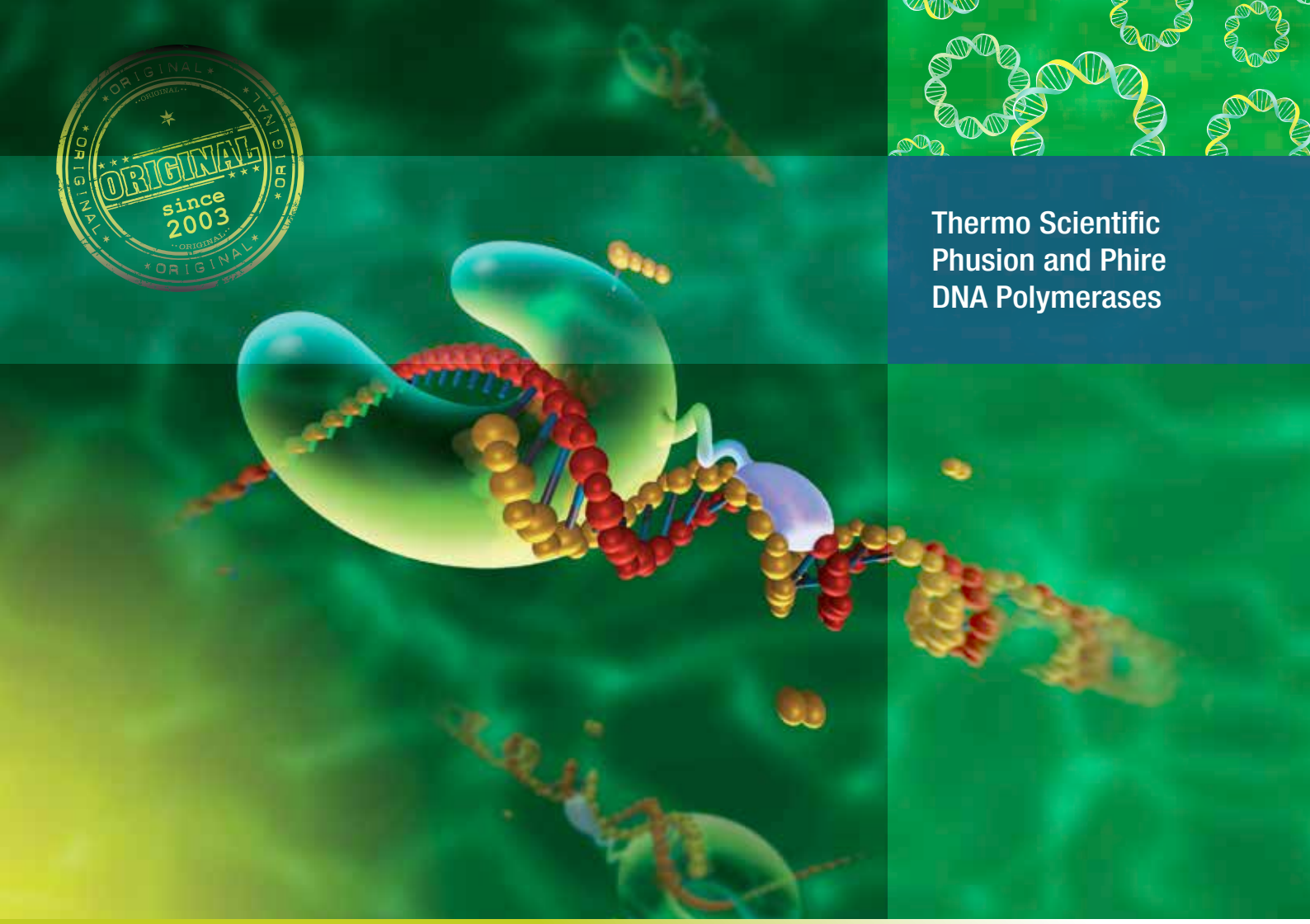


Thermo Scientific
Phusion and Phire
DNA Polymerases

Accurate, fast and powerful

Polymerases for better PCR

- Extremely accurate, fast and robust amplification
- Instant activation hot start technology
- Direct loading on gels
- dUTP tolerance and incorporation with Phusion U Polymerase

Thermo
SCIENTIFIC

Upgrade to the gold standard for high-fidelity PCR

Since their introduction in 2003, Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerases have established a new standard for high-fidelity PCR.[1,2]

Phusion High-Fidelity DNA Polymerase contains a DNA-binding domain fused to a *Pyrococcus*-like proofreading polymerase. Due to this unique fusion technique, Phusion DNA Polymerases generate PCR products with accuracy and speed unattainable with a single enzyme, even with difficult templates. In addition, Phusion DNA Polymerases are tolerant of various inhibitors allowing for robust amplification of PCR products with minimal optimization. For hot start PCR, Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase is an ideal choice allowing extreme specificity and improved robustness.

The processivity* of Phusion DNA Polymerases is approximately 10-fold greater than that of *Pfu* DNA polymerase and twice that of *Taq* DNA polymerase. This extremely high processivity results in shorter extension times, more robust amplification and the ability to amplify long templates (up to 20 kb) in a fraction of time. Phusion DNA Polymerases also produce higher yields with lower enzyme amounts than traditional proofreading polymerases.

NEW

The new Thermo Scientific™ Phusion™ Green format is a combination of Phusion DNA Polymerase and 5x Green Reaction Buffer. The buffer includes a density reagent and two tracking dyes for direct loading of PCR products on gels. The green buffer does not interfere with the performance of Phusion DNA Polymerase and is compatible with downstream applications including DNA sequencing, ligation and restriction digestion.

Features

- Accuracy – the highest fidelity thermostable polymerase (52x *Taq*)
- Robustness – fewer reaction failures and minimal optimization
- Speed – increased processivity allows shorter reaction times (extension 15-30 s/kb)
- High yields – increased product yields with minimal enzyme amounts (0.5-1 U/50 µL reaction)
- Specificity – unique hot start technology with zero-time reactivation reduces non-specific amplification and primer degradation
- Direct loading of PCR product on gels – Phusion Green and Phusion Green Hot Start II DNA Polymerases have loading dyes included into reaction buffers

Applications

- High-fidelity PCR
- Fast PCR
- Hot start PCR
- Long range PCR (up to 20 kb)
- High-throughput PCR

1. D.G. Gibson *et al.*, (2008) Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319, 1215-1220.
2. D.G. Gibson *et al.*, (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329, 52-56.

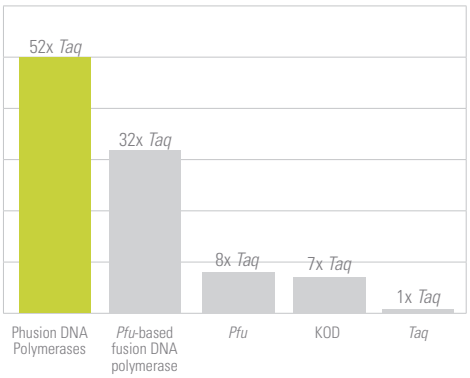
* Processivity measures the number of nucleotides the enzyme can incorporate into a growing DNA strand at one binding event during the extension step.

Extreme fidelity

In many molecular biology applications including cloning, site-directed mutagenesis and DNA translation, it is crucial to preserve the accurate DNA sequence during PCR amplification. An incorrectly incorporated nucleotide may change the respective codon and result in the addition of the wrong amino acid during translation. This, in turn, can affect folding and functional properties of the protein. Alternatively, deletion of a single nucleotide can destroy the correct reading frame.

Phusion DNA Polymerases have the highest fidelity of any available thermostable polymerase. The error rate of Phusion DNA Polymerase as determined by a modified *lac*I-based method[3] is approximately 50-fold lower than that of *Taq* DNA polymerase and six-fold lower than that of *Pfu* DNA polymerase (see graph below).

The low error rate of Phusion DNA Polymerase was recently confirmed in studies using 454 sequencing[4] and Illumina sequencing methods[5] (Table 1).



▲Relative fidelity values of different DNA polymerases. Fidelity = 1 / error rate.

	KOD (%)	Phusion HF (%)	Pt *Taq* (%)	Expand HF (%)	FastStart HF (%)	Sequal Prep Long (%)	*Pfu* Ultra HF (%)
Overall error rate[a]	0.21	0.11	0.34	0.25	0.23	0.29	0.23
Insertions	0.10	0.07	0.14	0.11	0.11	0.11	0.12
Deletions	0.06	0.02	0.08	0.07	0.05	0.06	0.05
Substitutions	0.01	0.01	0.07	0.04	0.03	0.07	0.01
Dots or Dot[b]	0.04	0.01	0.05	0.04	0.04	0.05	0.05

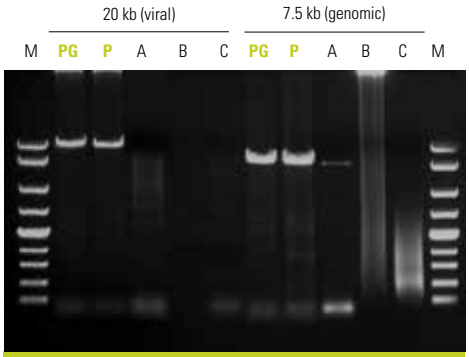
▲**Table 1.** Error rates determined by 454 sequencing for seven different DNA polymerases following PCR amplification of four different exons from the human TP53 oncogene. The clonal TP53 plasmid was used as a starting template.

a Error rate, number of errors (miscalled bases, inserted or deleted bases) divided by total number of bases.
b Dots or Dot, three successive negative flows during 454 sequencing.
Table reprinted with the permission from the corresponding author and the journal editor.

3. B.F. Frey & B. Suppmann (1995) Demonstration of the Expand PCR System's greater fidelity and higher yields with a *lac*I-based fidelity assay. *Biochemica* 2, 34-35.
4. Vandenbroucke *et al.* (2011) Minor variant detection in amplicons using 454 massive parallel pyrosequencing: experiences and considerations for successful applications. *Biotechniques.* 53:167-177.
5. Kinde *et al.* (2011) Detection and quantification of rare mutations with massively parallel sequencing. *PNAS.* 108(23):9530–9535.

Successful amplification of long targets

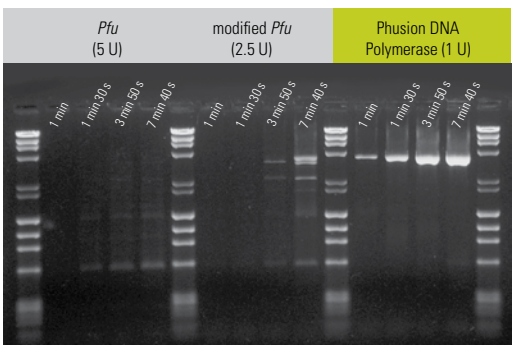
Phusion DNA Polymerases are the ideal choice for amplification of long templates. Extremely high enzyme processivity allows amplification of the widest variety of template sizes. Amplicons up to 20 kb are produced with high yields, short cycling times and higher fidelity.



▲**Superior yields of long PCR products**
A 20 kb fragment from λ DNA and 7.5 kb fragment from human genomic DNA was amplified with Phusion DNA Polymerases and proofreading DNA polymerases from other suppliers.

Robust amplification with less enzyme

Due to the unique structure of the enzyme, Phusion DNA Polymerases are highly efficient. When compared to conventional polymerases, significantly fewer units of the enzyme are required for any PCR amplification. Speed and efficiency result in high product yields in minimal time. In addition, Phusion polymerases are highly robust minimizing the need for reaction optimization.



▲**Less enzyme – superior yield**
A 3.8 kb fragment from human beta globin gene was amplified with three different DNA polymerases. Phusion DNA Polymerase was able to amplify the 3.8 kb genomic fragment with a combined annealing and extension step of only 1 minute, thus being significantly faster than the two other polymerases tested. A single unit of Phusion DNA Polymerase produced higher yields than 2.5 or 5 units of the *Pfu* DNA polymerases.

Extreme specificity hot start PCR

Phusion Hot Start II High-Fidelity DNA Polymerase is an extremely accurate hot start DNA polymerase. It combines the Phusion DNA Polymerase and a reversibly bound, specific Affibody™ ligand. The Affibody ligand inhibits the activity of the DNA polymerase at room temperature and thus prevents the amplification of nonspecific products. The reaction set-up can be done at room temperature enabling its use in high throughput robotics.

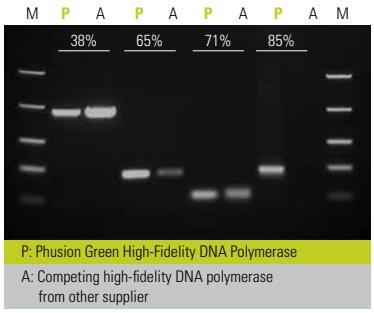
The Affibody ligand also inhibits the 3´→5´ exonuclease activity of the polymerase, preventing degradation of primers and template DNA during reaction set-up. At polymerization temperatures, the ligand is released, rendering the polymerase fully active. Phusion Hot Start II DNA Polymerase does not require a separate activation step in the PCR protocol as it is immediately reactivated at high temperatures.

▲ Thermo Scientific Phusion Hot Start II DNA Polymerase provides extreme specificity and abundant yields

Five proofreading hot start DNA polymerases from major suppliers were used to amplify 1.7-2.3 kb fragments from human genomic DNA. Phusion Hot Start II DNA Polymerase provided high yields of specific products whereas all other enzymes delivered zero or low yields, with some also amplifying non-specific products.

Robust amplification even with high GC content templates

Optimized buffer system in synergy with the high enzyme processivity enables Phusion DNA Polymerase to amplify a broad range of DNA templates with different sequence contexts. Efficient high-fidelity amplification has been demonstrated with difficult-to-amplify targets including those with 85% GC content.

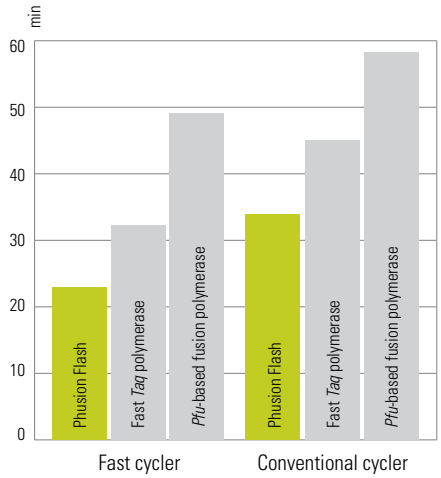


◀ Robust amplification of DNA fragments

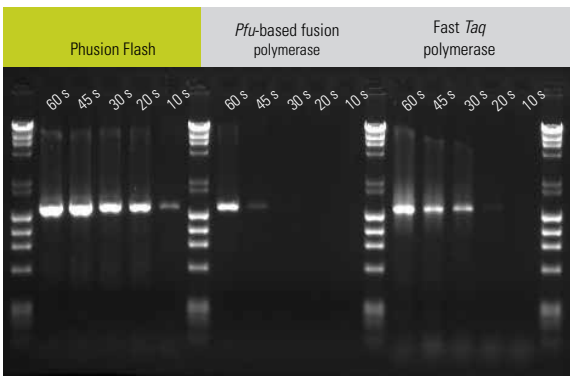
Four DNA fragments of different GC content were amplified. Phusion Green DNA Polymerase produced all four amplicons with high yields. In contrast, a competing high-fidelity DNA polymerase was not able to efficiently produce GC-rich product.

Fast PCR with high fidelity

Phusion DNA Polymerases incorporate more nucleotides per binding event as compared to other polymerases. This high processivity allows extremely short extension times and consequently reduced protocol times. Shortest protocol times can be achieved with Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix, a product developed specifically for fast PCR.



▲ Shorter PCR run times with Thermo Scientific Phusion Flash Master Mix



▲ Extreme speed and high yields with Thermo Scientific Phusion Flash Master Mix

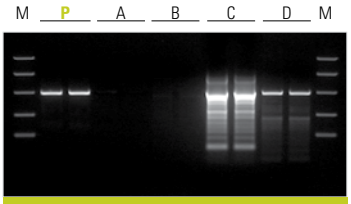
A 1.5 kb human cathepsin K gene was amplified with three different polymerases using varying extension times (10-60 s) with Thermo Scientific™ Piko™ Thermal Cycler. Only Phusion Flash Master Mix was able to amplify the 1.5 kb gene with extremely short extension times of 10 and 20 s. It also produced superior yields of specific product compared to other enzymes tested.

Engineered for dUTP-containing templates and dUTP incorporation

Thermo Scientific™ Phusion™ U Hot Start DNA polymerase is enabled to overcome an important limitation to the use of proofreading enzymes – it is tolerant to dUTP present in DNA templates and is able to incorporate dUTP.

A proprietary mutation developed in the so called dUTP binding pocket of Phusion enzyme allowed us to create the Phusion U Hot Start DNA Polymerase, a high fidelity polymerase that can work in such important applications as amplification of bisulfite-converted DNA or carry-over contamination control.

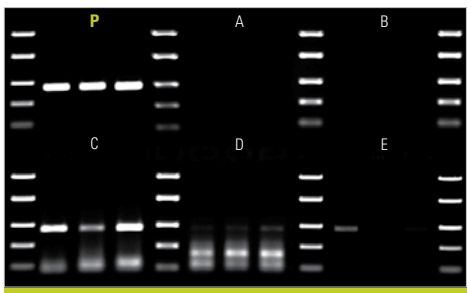
Phusion U Hot Start DNA polymerase retains all powers of Phusion family enzymes – great accuracy, speed, ability to amplify long amplicons up to 20 kb and a high specificity with Affibody® ligand-based hot start. It is also available with a Green buffer or in master mix format to further save time on pipetting.



P: Phusion U Hot Start DNA Polymerase
A: Proofreading fusion-type polymerase
B-D: Uracil-tolerant proofreading DNA polymerases from other suppliers

▲ Thermo Scientific™ Phusion U Polymerase incorporates dUTP into PCR product
2 kb fragment from 50 ng human genomic DNA amplified in the presence of dUTP (0.2 mM) according to recommendations of different suppliers of proofreaders with dUTP incorporation ability.

Highest yields and specificity with bisulfite-converted DNA



P: Phusion U Hot Start DNA Polymerase
A: Proofreading fusion-type polymerase
B-D: Uracil-tolerant proofreading DNA polymerases from other vendors
E: Hot start *Taq* DNA polymerase

◀ Thermo Scientific™ Phusion U Polymerase efficiently amplifies bisulfite-treated DNA
Five proofreading DNA polymerases and hot start *Taq* polymerase were used to amplify 798 bp fragment of bisulfite-treated human genomic DNA. Phusion U Hot Start DNA Polymerase provided high yields of specific products whereas all other enzymes delivered zero or lower yields, with some also amplifying non-specific products.

Features

- Accuracy – extremely high-fidelity DNA polymerase
- Uracil tolerance – engineered to amplify dUTP-containing templates and incorporate dUTP
- Specificity – hot start for no primer-dimers and non-specific bands
- Speed – short extension times (15-30 s/kb)
- Direct loading of PCR products on gels

Applications

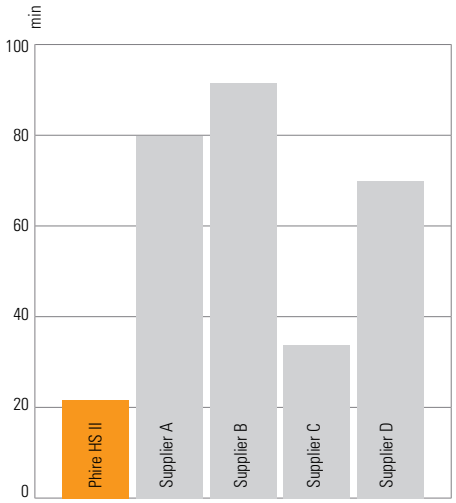
- Amplification of bisulfite-converted DNA
- Amplification of damaged or aged DNA
- Carry over contamination control
- Uracil-excision based (USER) cloning methods

Hot start PCR with improved speed, yields and amplicon length

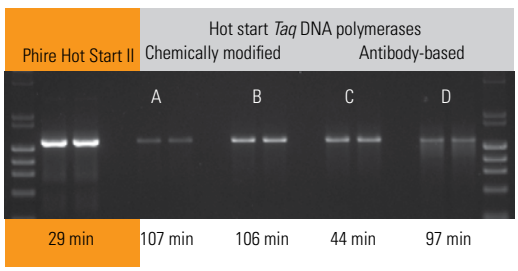
Thermo Scientific™ Phire™ Hot Start II DNA Polymerase incorporates a dsDNA-binding domain that allows short extension times (10-15 s/kb), improves yields, and increases fidelity two-fold compared to *Taq* DNA polymerase. In addition, the unique hot start technology allows complete reactivation of the enzyme in "zero-time" at standard cycling temperatures. This combination of features makes Phire Hot Start II DNA Polymerase an ideal solution for routine and high-throughput PCR applications.

Phire Green Hot Start II DNA Polymerase is supplied with 5x Phire Green Reaction Buffer that includes a density reagent and two tracking dyes for direct loading of PCR products on gels.

Both Phire and Phire Green Polymerases deliver superior performance in conventional and fast cycling thermal cyclers such as the Piko Thermal Cycler.



▲ Complete PCR cycling in less than half the time
A 600 bp fragment from human genomic DNA was amplified with five different hot start DNA polymerases. With Phire Hot Start II DNA Polymerase, the PCR protocol was completed up to four times faster than with *Taq* DNA polymerases utilizing chemically modified or antibody-based hot start technologies (suppliers A-D). Green buffer further reduces experimental time by eliminating one pipetting step and allowing for direct loading on gel.



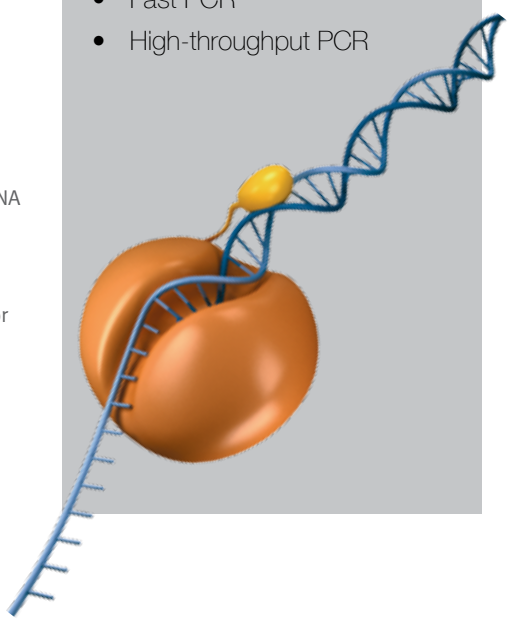
◀ Superior yields in significantly shorter time
A 1.5 kb fragment from the human cathepsin K gene was amplified with five different hot start DNA polymerases. Phire Hot Start II DNA Polymerase amplified high amounts of specific PCR product in just 29 minutes. In contrast, the PCR protocols for hot start *Taq* DNA polymerases from four major suppliers (A-D) were substantially longer and resulted in lower product yields.

Features

- Quick hot start – no reactivation step
- Fast – amplify 4x faster than with hot start *Taq*
- Robust – minimal reaction optimization due to high inhibitor tolerance
- High yields of PCR products
- Longer PCR products – amplify significantly longer DNA fragments than with any hot start *Taq*
- Direct loading of PCR product on gels – Phire Green Hot Start II DNA Polymerase has loading dyes included into reaction buffer

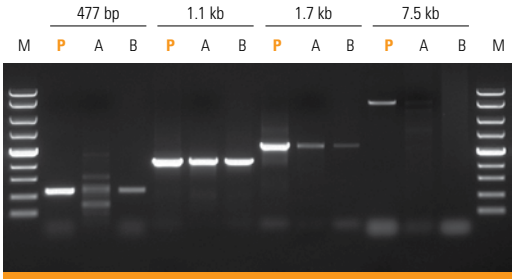
Applications

- Hot start PCR
- Routine PCR
- Fast PCR
- High-throughput PCR



Thermo Scientific Phire Green reaction buffer – superior enzyme performance and ultimate convenience

▲ **1x reaction mixture containing Thermo Scientific Phire Green Reaction Buffer.**
A – unseparated in a well
B – blue and yellow dyes following electrophoresis



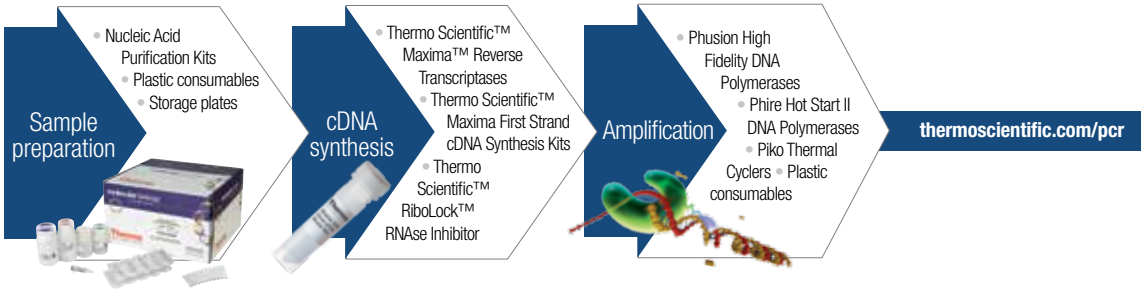
▲ **Thermo Scientific Phire Green Hot Start II DNA Polymerase amplifies longer fragments than any hot start *Taq***
Five human genomic DNA fragments of different lengths were amplified with three different hot start DNA polymerases. Phire Green Hot Start II DNA Polymerase produced all five amplicons with high yields. The competing hot start *Taq* DNA polymerases produced significantly lower yields and failed to amplify the 7.5 kb fragment.

Enzyme characteristics and formats

		Phusion U Hot Start DNA Polymerase	Phusion High-Fidelity DNA Polymerase	Phusion Hot Start II High-Fidelity DNA Polymerase	Phusion Flash High-Fidelity DNA Polymerase	Phire Hot Start II DNA Polymerase
CHARACTERISTICS	Blunt or 3'A end	Blunt	Blunt	Blunt	Blunt	Blunt
	Target length, genomic/phage DNA	≤ 20 kb	≤ 16/20 kb	≤ 16/20 kb	≤ 16/20 kb	≤ 7.5/20 kb
	Hot start	Yes	No	Yes	Yes	Yes
	Recommended extension time	15-30 s/kb	15-30 s/kb	15-30 s/kb	15 s/kb	10-15 s/kb
	Fidelity vs. *Taq*	25x	52x	52x	25x	2x
	dUTP tolerance	Yes	No	No	No	No
AVAILABLE AS	Enzyme[1]	✓	✓	✓	–	✓
	Green Buffer[2]	✓	✓	✓	–	✓
	Master mix[3]	✓	✓	–	✓	–
	Complete kit[4]	–	✓	–	–	–

1. Enzyme: DNA polymerase, buffer(s), DMSO and $MgCl_2$
2. Green Buffer: DNA polymerase supplied with a Green buffer that includes density reagent and two tracking dyes for direct PCR product loading on gel
3. Master mix: 2x master mix
4. Complete kit: All the necessary PCR reaction components including control template and primers

Everything for your Thermo Scientific PCR workflow

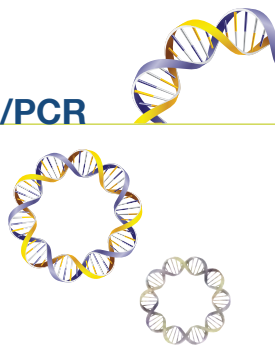


Order details

Product Description	Quantity	Cat.No.
Phusion High-Fidelity DNA Polymerases, Master Mixes and Kits		
Phusion High-Fidelity DNA Polymerase	100 U	F-530S
	500 U	F-530L
Phusion Green High-Fidelity DNA Polymerase	100 U	F-534S
	500 U	F-534L
Phusion High-Fidelity PCR Master Mix with HF Buffer	100 × 50 µL rxns	F-531S
	500 × 50 µL rxns	F-531L
Phusion High-Fidelity PCR Master Mix with GC Buffer	100 × 50 µL rxns	F-532S
	500 × 50 µL rxns	F-532L
Phusion Flash High-Fidelity PCR Master Mix	100 × 20 µL rxns	F-548S
	500 × 20 µL rxns	F-548L
Phusion High-Fidelity PCR Kit	125 × 20 µL rxns	F-553S
	500 × 20 µL rxns	F-553L
Phusion Hot-Start II High-Fidelity DNA Polymerase	100 U	F-549S
	500 U	F-549L
Phusion Green Hot Start II High-Fidelity DNA Polymerase	100 U	F-537S
	500 U	F-537L
Phusion RT-PCR Kit	20 rxns	F-546S
	100 rxns	F-546L
Phusion Site-Directed Mutagenesis Kit	20 rxns including 10 control rxns	F-541
Phusion U Hot Start DNA Polymerases and Master Mix		
Phusion U Hot Start DNA Polymerase	100 U	F-555S
	500 U	F-555L
Phusion U Green Hot Start DNA Polymerase	100 U	F-556S
	500 U	F-556L
Phusion U Hot Start PCR Master Mix	100 x 50 µL rxns.	F-533S
	500 x 50 µL rxns.	F-533L
Phire Hot Start II DNA Polymerase		
Phire Hot Start II DNA Polymerase	200 × 50 µL rxns (or 500 × 20 µL rxns)	F-122S
	1000 × 50 µL rxns (or 2500 × 20 µL rxns)	F-122L
Phire Green Hot Start II DNA Polymerase	200 × 50 µL rxns (or 500 × 20 µL rxns)	F-124S
	1000 × 50 µL rxns (or 2500 × 20 µL rxns)	F-124L

In addition to Phusion and Phire PCR enzymes and kits, the Thermo Scientific™ PCR portfolio includes PCR instruments, reaction vessels and alternative DNA polymerases for various applications.

- Learn more at **thermoscientific.com/PCR**



> "After realizing that we could get the same number of cycles in roughly a quarter of the time (and at only slightly higher per unit cost), we changed exclusively to Phusion [Polymerase]."
>
> Matt W. Ford, PhD student,
> Department of Biological Sciences,
> Idaho State University, USA.

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